PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MÜLLER, Theodor
OK PAT AG
Chamerstrasse 50
CH-6300 Zug
SUISSE

EINGEGANGEN 0 8. März 2004

PCT

WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	04.03.2004
Applicant's or agent's file reference: E1103-WO	**REPLY DUE** **within 3 month(s)** from the above date of mailing
International application No.: PCT/CH 03/00153	International filing date *(day/month/year)*: 05.03.2003
Priority date *(day/month/year)*: 07.03.2002	
International Patent Classification (IPC) or both national classification and IPC: C12N9/10, C12N9/10	
Applicant: EIDGENÖSSISCHE TECHNISCHE HOCHSCHULE ZÜRICH et al.	

Termin: 4.6.04
Eintrag in Fristenliste durch: Vsm

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is:

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer
Formalities officer (incl. extension of time limits)
Sülberg, A.
Telephone No. +49 89 2399-7548

WRITTEN OPINION

International application No. PCT/CH 03/00153

I. **Basis of the opinion**

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-13 as originally filed

Claims, Numbers

1-13 as originally filed

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

WRITTEN OPINION International application No. PCT/CH 03/00153

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 9-13

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an International preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no International search report has been established for the said claims Nos. 9-13

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the Standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	
Inventive step (IS)	Claims	1-8
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

No international search report was established for claims 9-13. Said claims are therefore not subject to the preliminary examination as set forth under Rule 66.1 (e) PCT.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application presents an *E. coli* expression system for production of N-glycosylated proteins. The campylobacter jejuni glycosylation machinery (pgl) was transferred into E. coli for this purpose. Recombinant AcrA protein was produced and glycosylation verified by mass spectroscopy.

2. Reference is made to the following documents:

   D1: SZYMANSKI C M ET AL: "EVIDENCE FOR A SYSTEM OF GENERAL PROTEIN GLYCOSYLATION IN CAMPYLOBACTER JEJUNI" MOLECULAR MICROBIOLOGY, BLACKWELL SCIENTIFIC, OXFORD, GB, vol. 32, no. 5, 1999, pages 1022-1030, XP008012013 ISSN: 0950-382X

   D2: WACKER M ET AL: "N-linked glycosylation in Campylobacter jejuni and its FUNCTIONAL TRANSFER INTO E.COLI" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 298, 29 November 2002 (2002-11-29), pages 1790-1793, XP002225920 ISSN: 0036-8075

2. **Priority**

Since the priority document pertaining to the present application is not yet available to the IPEA, this Written Opinion has been drawn up considering the

priority date (7. 03. 2002) as valid. D2 (Wacker *et al.*) has been published between the priority date and the filing date of the present application. Thus, said document is not considered to constitute prior art in the meaning of Rule 64(1)(b) PCT. However, if it turns out that the effective date of the claimed subject-matter is not the priority date, then D2 will become relevant to assess whether the present application satisfies the criteria set forth in Art. 33(2) and (3) PCT.

2. **Novelty** (Art. 33 (2) PCT)

Claims 1-8 appear to be novel over the prior art cited in the ISR.

3. **Inventive Step**(Art. 33 (3) PCT)

3.1 D1 discloses the pgl locus in *C. jejuni* and its individual genes, including pglB as oligosaccharide transferase (Fig. 1B, table 1). The pgl genes were introduced into E. coli, which resulted in altered LPS cores and reactivity to O:23/O:36 serum (Fig. 2; p. 1024, left-hand column, paragraph 3). This result shows that the E. coli LPS had the C. jejuni oligosaccharide pattern upon transformation with the pgl locus.

3.2 Although D1 does not provide evidence for N-glycosylation, the E. coli proteins were likely to be N-glycosylated, since D1 uses the same gene cluster as the present application, i.e. the E. coli transfected with pRY407 would be suitable for N-linked glycosylation.

3.3 D1 does not disclose introduction of a foreign gene into E. coli and its subsequent N-glycosylation with the C. jejuni pattern. However, it appears that the skilled person would immediately regard as evident that if the LPS cores were changed this would affect not only endogenous but also exogenous genes.
Therefore, in view of D1 and the general common knowledge, the subject-matter of claims 1-8 is considered not to involve an inventive step in the sense of Art. 33 (3) PCT.

4. **Clarity/Sufficiency of Disclosure** (Art. 6/5 PCT)

4.1 Claims 1-8 attempt to define the subject-matter in terms of the result to be

achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added (cf PCT Guidelines III 4.7).
Moreover, sufficient disclosure is lacking (Art. 5 PCT) because the claims broadly extend to *any* metabolic apparatus capable of carrying out N-glycosylation, which is in contrast to the disclosure of only one single prokaryotic machinery (C. jejuni) that has been transferred into E. coli.
Selecting other metabolic systems for transfer into E. coli would require extensive testing with regards to the functionality of system (i.e. whether the system really produces glycosylated proteins), which amounts to an undue burden for the skilled person. It might indeed be very difficult to find any other bacterial glycosylation system suitable for the desired purpose when considering the following statement in D2: "To our knowledge, a general N-glycosylation system very similar to the one found in eukaryotes has not been described in other bacteria, and the C. jejuni genome is the only bacterial genome sequenced to date that harbors a gene that encodes a protein with strong sequence homology to a eukaryotic oligosaccharyltransferase component." (p. 1793, left-hand column, last paragraph).

4.2 If the difference between D1 and the present application is considered to be the establishment of N-linked glycosylation, there must be a relevant essential technical feature on which the said difference is based. It appears, however, neither the claims nor the description clearly define the said feature. Example 1, which apparently represents the only working example (although not presenting any data), refers to the OTase of *C.jejuni* but fails to specify the difference of the OTase to the known OTase of D1. There seems to be no guidance on which part of the C. jejuni genome needs to be transferred to achieve N-glycosylation. Thus, along these lines, the skilled person is not able to carry out the invention and the application as a whole appears to lack sufficiency of disclosure in the sense of Art. 5 PCT.

4.3 It is noted that example 1 refers to the procedures disclosed in D2, which was published after the priority date. D2 can, however, not be consulted to establish sufficiency of disclosure at the priority date.